Platelet-rich concentrate in serum free medium enhances osteogenic differentiation of bone marrow-derived human mesenchymal stromal cells

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ABSTRACT

Previous studies have shown that platelet concentrates used in conjunction with appropriate growth media enhance osteogenic differentiation of human mesenchymal stromal cells (hMSCs). However, their potential in inducing osteogenesis of hMSCs when cultured in serum free medium has not been explored. Furthermore, the resulting osteogenic molecular signatures of the hMSCs have not been compared to standard osteogenic medium. We studied the effect of infrequent supplementation (8-day interval) of 15% non-activated platelet-rich concentrate (PRC) in serum free medium on hMSCs proliferation and differentiation throughout a course of 24 days, and compared the effect with those cultured in a standard osteogenic medium (OM). Cell proliferation was analyzed by alamar blue assay. Gene expression of osteogenic markers (Runx2, Collagen1, Alkaline Phosphatase, Bone morphogenetic protein 2, Osteopontin, Osteocalcin, Osteonectin) were analyzed using Q-PCR. Immunocytochemical staining for osteocalcin, osteopontin and transcription factor Runx2 were done at 8, 16 and 24 days. Biochemical assays for the expression of ALP and osteocalcin were also performed at these time-points. Osteogenic differentiation was further confirmed qualitatively by Alizarin Red S staining that was quantified using cetylpyridinium chloride. Results showed that PRC supplemented in serum free medium enhanced hMSC proliferation, which peaked at day 16. The temporal pattern of gene expression of hMSCs under the influence of PRC was comparable to that of the osteogenic media, but at a greater extent at specific time points. Immunocytochemical staining revealed stronger staining for Runx2 in the PRC-treated group compared to OM, while the staining for Osteocalcin and Osteopontin were comparable in both groups. ALP activity and Osteocalcin/DNA level were higher in the PRC group. Cells in the PRC group had similar level of bone mineralization as those cultured in OM, as reflected by the intensity of Alizarin red stain. Collectively, these results demonstrate a great potential of PRC alone in inducing proliferation of hMSCs without any influence from other lineage-specific growth media. PRC alone has similar capacity to enhance hMSC osteogenic differentiation as a standard OM, without changing the temporal profile of the differentiation process.

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